Avian Leukosis Virus (P27) Antigen ELISA Kit

ALV P27 Ag Test

Product Number: E21161
Product Unit: 1 plate-96wells, 2 plates-192wells, 5 plates-480wells,
for 10plates, 2 units of 5plates will be provided

Manufacturer Information
Ring Biotechnology Co., Ltd
Add: Building 3, Zhongtongtai TechnoPark, No. 11, Kechuang 14th St, Beijing 100176, CHINA
E-mail: info@ringbio.com Web: www.ringbio.com
Tel: +86-10-56267496 +86-18600362934

1. Introduction

Avian sarcoma leukosis virus (ASLV) is an endogenous retrovirus that infects and can lead to cancer in chickens; experimentally it can infect other species of birds and mammals. ASLV replicates in chicken embryo fibroblasts, the cells that contribute to the formation of connective tissues. Lymphoid leukosis is the most common form of this disease and with typical presentation of gradual onset, persistent low mortality, and neoplasia of the bursa. Subgroups A, B, E and J are the major subgroups of avian leukosis virus (ALV) infecting chickens. ALV infection has become endemic in many countries and has a significant negative effect on the poultry industry.

As the capsid protein, P27 is the group-specific antigen of ALV and has many viral antigen sites, which enable the easy detection of the virus itself, and based on which ELISA or lateral flow immunoassay were further developed for laboratory testing purpose.

2. Description of Test

The current ALV-Ag P27 ELISA kit is designed to detect P27 protein in various chicken samples. The 96well microtiter plate was precoated with a P27 protein specific monoclonal antibody. During testing, samples are added into the microplate wells, in which the precoated antibody will capture the ALV in sample and formed antigen-antibody complex. None specific antigens are discarded by a washing step. Then another anti-P27 monoclonal antibody conjugate labeled with horseradish peroxidase (HRP) is added into each well, and further forms antibody-antigen-antibody complexes. After another washing step to remove unreacted conjugate, substrate is added and a blue color will be developed if P27 antigen is present. The enzyme reaction is stopped and the OD650nm value is measured. The measured intensity is positively proportional to the amount of P27 antigen present in the sample.

This ELISA kit can be used to detect ALV in **egg albumen**, **meconium, virus culture** and **cloacal swabs**.

3. Precautions

- Store the kit at 2-8°C,Check the lot number and expiration date before use.
- Bring the test kit to room temperature(18-26°C) before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

4. Limitations of Test

This ELISA kit is currently designed for veterinary use. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned

purpose, please contact us for help.

5. Reagent Provided

This kit has different sizes. Each size contains the following different items.

Item No.	Description	96wells	192wells	480wells
1	Microplate pre-coated with antibody	1 X 96 wells	2 X 96 wells	5 X 96 wells
2	Positive Control	1 X 1 ml	1 X 1 ml	2 X 2 ml
3	Negative Control	1 X 1 ml	1 X 1 ml	2 X 2 ml
4	Enzyme Conjugate	1 X 25 ml	1 X 25 ml	1 X 60 ml
5	TMB Substrate	1 X 25 ml	1 X 25 ml	1 X 60 ml
6	Stop Solution	1 X 20 ml	1 X 20 ml	1 X 40 ml
7	25X Wash Buffer	1 X 30 ml	1 X 30 ml	1 X 60 ml
8	Sample Buffer	1 X 30 ml	1 X 30 ml	1 X 60 ml
9	Kit Instruction	1 set	1 set	1 set

6. Instrument Required

- ELISA reader with 650nm
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- **Wash buffer**: dilute the 25X Wash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 1ml 25X Wash buffer + 24ml deionized water
  The diluted wash buffer can be stored at 2-8°C for 3 days.
  No other reagent is required. Please remember to return all kit component to room temperature before use.

8. Sample Preparation

- **Egg albumen or virus culture:** use for assay directly without any pre-treatment.
- **Cloacal swab / Meconium:** dissolve or extract with **0.01M pH7.2-7.4 PBS**. For example, 100ul sample + 500ul -1000ul PBS, repeated freezing and thawing is recommended to improve the ALV antigen extraction.

9. Assay Procedure

1) Make sure the kit and all test samples are returned to room temperature(18-26°C) before use. Shake each reagent gently before adding into the well.
2) Open the kit, read the kit instruction carefully to make sure all technical points are understood clearly.
3) Take the microplate from the zip-bag, and take needed microwells, store the rest into the zip-bag. Make marks of the plate layout. Running the test in duplicated wells is recommended to minimize operational error.
4) **Add Positive control:** add **100ul positive control** into two wells.
5) **Add Negative control:** add **100ul negative control** into another two wells.
6) **Add Sample:** add **100ul prepared sample** into the wells.
7) **Incubation:** cover the plate with plate cover and incubate at **25 °C for 60min.**

8) **Washing:** pour the liquid out from the wells and wash with wash buffer (300ul per well) for 4-5 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.

9) **Add enzyme conjugate:** add **100ul of enzyme conjugate** into each well. Cover the plate again and then incubate at **25 °C for 60min.**

10) **Washing:** repeat the washing step again.

11) **Add TMB substrate:** add the **100ul TMB substrate** into each well. Cover the plate again and then incubate at **25 °C for 15min**. Color reaction will occur in the plate.

12) **Stop the reaction:** add **50ul stop solution** into each well, the color will turn yellow from blue.

13) **Read the plate:** using ELISA reader to read the plate at **650nm**.

10. Result Determination

If mean **OD of negative control < 0.15**, and **mean OD of positive control – mean OD of negative control ≥ 0.25**, the test is valid. Otherwise, please run the analysis again with new kit.

1) Calculation of cut-off value S/P:

$$\frac{\text{Mean OD of Sample – Mean OD of Negative Control}}{\text{Mean OD of Positive Control – Mean OD of Negative Control}} = \text{S/P}$$

2) Criteria of Positive and Negative results.

Positive: **S/P ≥ 0.2** **Negative**: **S/P < 0.2**

11. Performance of Test

According to field test with over 500 samples, the sensitivity of the kit is 96.8%, and the specificity of the kit is 99.7%. This kit is approved by the ministry of agriculture.

12. Storage and expiration

The kit shall be store at 2-8°C, avoid direct sunlight.

The valid period is 12 months.

13. References

(1) https://en.wikipedia.org/wiki/Avian_sarcoma_leukosis_virus

(2) Yun B, Li D, Zhu H, et al. Development of an antigen-capture ELISA for the detection of avian leukosis virus p27 antigen.[J]. Journal of Virological Methods, 2013, 187(2):278-283.